PATENT COOPERATION TREATY

PCT

INTERNATIONAL-TYPE SEARCH REPORT

(PCT Article 15.5)

National application No.	Country or Office of filing	Applicant's or agent's file reference
0300567-5	SE	PU 0318-SE

Filing date (day/month/year)	(Earliest) Priority Date (day/month/year)
28 February 2003	

Applicant

AMERSHAM BIOSCICENCES AB

Date of request for international-type search	International-type search request No.
28 February 2003	SE 03/00134

This international-type search report has been prepared by this International Searching Authority and is transmitted to the applicant.

This international-type search report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] Certain claims were found unsearchable (See Box I).

2. [ ] Unity of invention is lacking (See Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international-type search was carried out on the basis of the sequence listing

   [ ] filed with the international application.

   [ ] furnished by the applicant separately from the international application,

      [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.

   [ ] transcribed by this Authority.

INTERNATIONAL-TYPE SEARCH REPORT

Search request No.
SE 03/00134

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C07K 1/22
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C07D, C07C, C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-INTERNATIONAL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 0181365 A2 (SIGMA-ALDRICH CO.), 1 November 2001 (01.11.01)	18-28
A		1-17
	--	
X	WO 9422497 A1 (INSTITUT FÜR DIAGNOSTIKFORSCHUNG GMBH AN DER FREIEN UNIVERSITÄT BERLIN), 13 October 1994 (13.10.94)	20
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X	WO 9422492 A1 (INSTITUT FÜR DIAGNOSTIKFORSCHUNG GMBH AN DER FEIEN UNIVERSITÄT BERLIN), 13 October 1994 (13.10.94)	20
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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international-type search	Date of mailing of the international-type search report
28 August 2003	2003 -09- 03
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Eva Johansson/EÖ Telephone No. +46 8 782 25 00

Form PCT/ISA/201 (second sheet) (March 1993)

INTERNATIONAL-TYPE SEARCH REPORT

Search request No.
SE 03/00134

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0108406 A2 (F. HOFFMANN-LA ROCHE & CO.), 16 May 1984 (16.05.84)	20
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A	US 4877830 A (HEINZ DÖBELI ET AL), 31 October 1989 (31.10.89)	1-28
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A	US 4551271 A (ERICH HOCHULI), 5 November 1985 (05.11.85)	1-28
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				CA	2156620	A	13/10/94
				DE	4311023	A,C	06/10/94
				DE	59408142	D	00/00/00
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				ZA	8401395	A	31/10/84

B1

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A2

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(54) **Bifunctional chelating agents.**

(57) Novel homocysteine thiolactone bifunctional chelating agents useful for chelating radionuclides to produce a radiodiagnostic agent for use in in vivo imaging and a method for producing this chelating agent.

EP 0 108 406 A2

0108406

F. Hoffmann-La Roche & Co.
Aktiengesellschaft
Basel / Schweiz

4. Nov. 1983

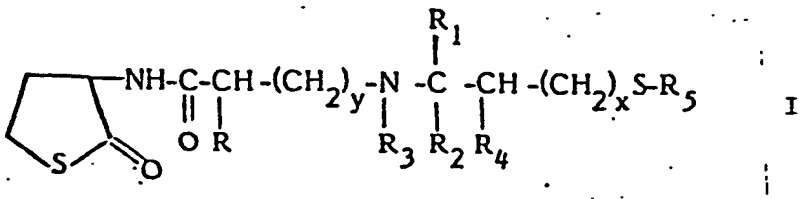
RAN 4090/141

Bifunctional chelating agents

Scintigraphy and similar radiographic techniques are finding increasing application in biological and medical research and diagnostic procedures. Scintigraphy involves the use of radiopharmaceuticals having a radioactive material which upon introduction into a biological subject, becomes localized in specific organs, tissue or skeletal material desired to be imaged. When so localized, traces, plates or scintiphotos of the distribution of the radioactive material may be made by various radiation detectors. The resultant distribution of the radioactive material in the organ or tissue in which it is localized can be used to detect the presence of aberations, pathological conditions or the like.

In preparing the radiopharmaceutical, radionuclide chelating agents are utilized which will act as a bridge to connect the radioactive material such as a radioactive metal and the material which will localize in the organ, or tissue to be imaged. In general, effective chelating agents are desired which will couple the radionuclides to the material which will localize in the organ to be imaged.

In accordance with this invention, it has been discovered that novel compounds of the formula:



I

wherein R is hydrogen or lower alkyl, R_1 and R_2 are individually hydrogen or lower alkyl or taken together form oxo; R_3 is an amino protecting group where R_1 and R_2 are individually hydrogen or lower alkyl;

R_3 is hydrogen when R_1 and R_2 taken together form oxo; R_4 is hydrogen or lower alkyl; R_5 is hydrogen or a thiol protecting group; x and y are integers from 0 to 2

are bifunctional chelating agents and as such can couple radionuclides to terminal amino containing compounds capable of localizing in an organ or tissue which is desired to be imaged. Hence, the compound of formula I can be used in preparing radiopharmaceuticals for in vivo diagnostic imaging.

The term "lower alkyl" as used throughout this application designates aliphatic saturated branched or straight chain hydrocarbon monovalent substituents containing from 1 to 7 carbon atoms such as methyl, ethyl, isopropyl, n-propyl, n-butyl, etc. The term "lower alkoxy" as used throughout this specification designates lower alkoxy substituents containing from 1 to 7 carbon atoms such as methoxy, ethoxy, isopropoxy, etc. The term "halogen" designates all four halogens such as chlorine, fluorine, iodine, or bromine.

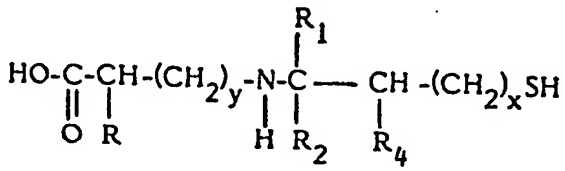
The term "aryl" as utilized herein designates a mononuclear aromatic hydrocarbon group which can be unsubstituted or substituted in one or more positions with a lower alkyl group such as phenyl or tolyl as well as polynuclear aromatic hydrocarbon groups which can be unsubstituted or substituted in one or more positions with a lower alkyl group such as napthyl, anthryl, phenanthryl, azulyl, etc. The preferred lower aryl group is phenyl. The term "aryl lower alkyl" designates aryl lower alkyl substituents where aryl and lower alkyl groups as defined above, particularly benzyl.

The term "lower alkanoyl" as used throughout this specification designates "lower alkanoyl" groups containing from 2 to 7 carbon atoms such as acetyl, propionyl, etc. The term "arylloweralkanoyl" designates monovalent aryl-

loweralkanoyl groups where aryl and lower alkanoyl ar defined as above with phenylacetyl being preferred. The term "aroyl" defines aroyl groups where the aryl group is defined as above with benzoyl being preferred.

As used herein, the term "thiol protecting group" includes all of the conventional groups which are commonly employed to protect the thiol moiety. Among these groups are included lower alkylaminocarbonyl such as ethylaminocarbonyl, loweralkanoylaminomethyl, aroylaminomethyl, t-butyl, triarylmethyl such as triphenylmethyl, aroyl such as benzoyl, aryloxycarbonyl such as phenoxycarbonyl, aryl-loweralkoxylcarbonyl, preferably arylmethoxycarbonyl such as benzyloxycarbonyl, lower alkoxycarbonyl such as t-butoxycarbonyl. Among the preferred lower alkanoylaminomethyl groups is aceteamidomethyl and among the preferred aroylaminomethyl is benzoylaminomethyl. The thiol protecting groups are removable by treatment with heavy metallic ions such as mercuric ions, techneticum ions, silver ions, as well as any of the radioactive metals which form the complex. Any of the conventional methods commonly employed in removing these thiol protecting groups can be utilized in accordance with this invention.

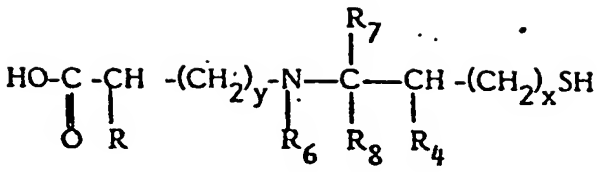
In accordance with this invention, the compound of formula I is prepared from a compound of the formula:



II

wherein R, R_1, R_2, R_3, R_4, x and y are as above.

When R_1 and R_2 are other than oxo, the nitrogen group in the compound of formula II is protected with a conventional amino protecting group to produce a compound of the formula:



II-A

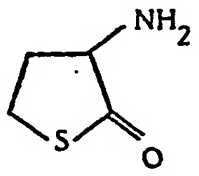
wherein x, y, R and R_4 are as above; R_6 is an amino protecting group; R_7 and R_8 are individually hydrogen or lower alkyl.

Any conventional method of converting a secondary amine to a protected amine can be utilized in converting the compound of formula II to the compound of formula II-A. Any of the conventional amino protecting groups which can be removed by conventional acid hydrolysis or catalytic hydrogenation can be utilized in this invention. Among the preferred amino protecting groups are included triarylmethyl such as trityl, arylloweralkoxycarbonyl such as benzyloxycarbonyl, lower alkoxycarbonyl such as t-butoxycarbonyl, aryl such as benzyl, etc. Any conventional method of preparing these protected amino groups can be utilized in accordance with this invention. Among these methods are reacting the compound of formula II where R_1 and R_2 are hydrogen or lower alkyl with the halide of the protecting group to be introduced into the compound of formula II. Any of the conditions conventional in such reactions can be utilized.

The compounds of formula II or II-A can be either free acids or the acid can be protected by esterification. The use of an ester increases the yield where either the compound of formulae II or II-A is reacted to introduce an amino protecting or a thiol protecting group. However, this ester group should be hydrolyzed before the compound of formulae II or II-A is reacted to form the compound of formula I. Any conventional method of ester hydrolysis can be used to form the free acid of formula I.

A thiol protecting group can be introduced if desired into the compound of formula II or II-A by conventional means. It has been found that best results as far as yields are achieved when the thiol group in the compound of formula II and formula II-A is protected with any of the groups hereinbefore mentioned. On the other hand, the reaction and production of the bifunctional chelate can be achieved without the use of a thiol protecting group.

The compounds of formulae II or II-A in their free acid form and when R_1 and R_2 are hydrogen or a lower alkyl, the amino group is protected with an amino protecting group and which may or may not contain a thiol protecting group can be converted to the compound of formula I by reaction with a compound of the formula:

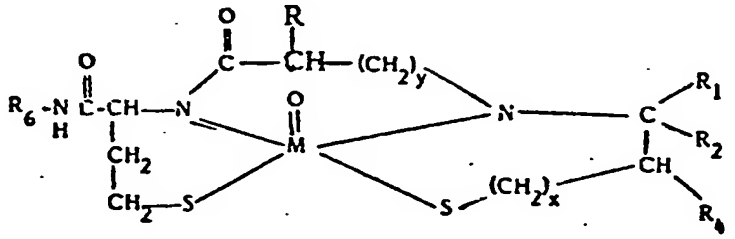


IV

This reaction is carried out by conventional amide formation. Any conventional means of reacting an organic carboxylic acid with an amine to form an amide can be utilized in this conversion to produce the compound of formula I. Generally this reaction is carried out in the presence of an amide condensation agent such as a loweralkylhalo formate, i.e. ethylchloroformate or dicyclohexyl carbodiimide. When utilizing the alkyl chloro formate method, the amide formation occurs by means of a mixed anhydride since the alkylchloroformate forms an anhydride with the compound of formula II-A which then reacts with the amine group on the compound of formula IV to form the amide of formula I. Any of the conditions conventionally used in reacting an organic acid with an amine to form an amide through the use of a loweralkylhaloformate can be utilized in carrying out this procedure. On the other hand, when a coupling agent such as dicyclohexylcarbodiimide is utilized, any of the conditions

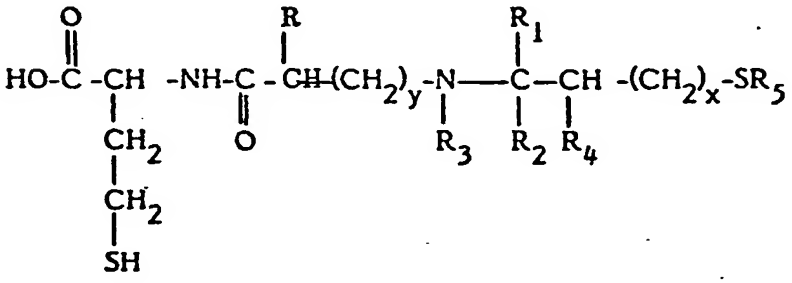
conventionally utilized with such coupling agent can be used to produce the compound of formula I.

The compound of formula I can be converted to a bifunctional anionic chelate of the formula:

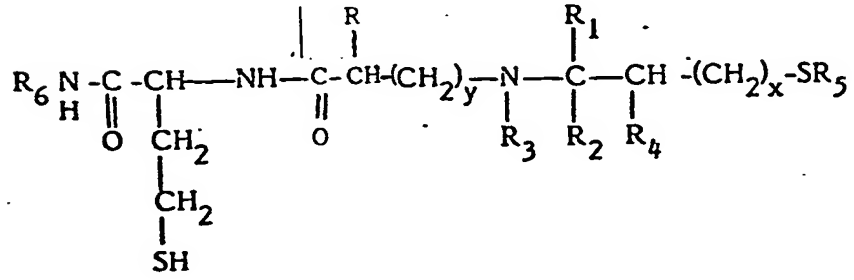


VI

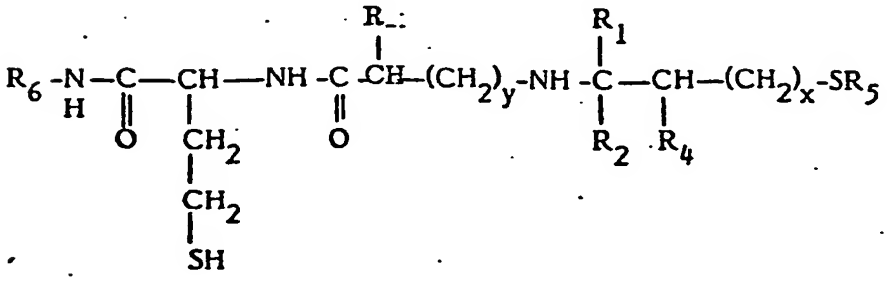
wherein R_6 is lower alkyl; R_1 and R_2 are individually hydrogen or lower alkyl or taken together form oxo; R_4, R, x and y are as above; M is a radioactive metal via the following intermediates:



VII



VIII



IX

wherein R, R_1, R_2, x, y, R_3, R_4, R_5 and R_6 are as above, with the proviso that R_3 is an amino protecting group when R_1 and R_2 are individually hydrogen or lower alkyl; and with the further proviso that R_3 is hydrogen when R_1 and R_2 taken together form oxo.

The compound of formula I is converted to the compound of formula VII by treating the compound of formula I with a base. Any conventional strong inorganic base such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, etc. can be utilized in carrying out this reaction. Any of the conditions conventional in hydrolysis with an alkali metal base can be used to carry out this reaction.

The compound of formulae I or VII can be converted to the compound of formula VIII by reacting either of these compounds with a compound of the formula:

$$R_6 - NH_2 \qquad X$$

where R_6 is as above.

The compound of formulae VII is reacted with the compound of formula X utilizing the same conditions described in connection with the reaction of the compound of formula IV with the compound of formulae II or II-A to produce the compound of formula I. In fact, any conventional method of condensing a lower alkyl amine with an organic carboxylic acid to produce an amide can be utilized in carrying out this reaction.

The compound of formula I can also be reacted with the compound of formula X to produce a compound of formula VIII. This reaction is carried out by mixing the compound of formula I and the compound of formula X together in an anhydrous inert organic solvent. Any conventional inert organic solvent can be utilized in carrying out this reaction with other solvents such as tetrahydrofuran being pre-

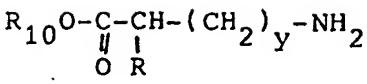
ferred. In carrying out this reaction, room temperature and atmospheric pressure are generally utilized.

In the next step, the compound of formula VIII where R_3 is an amino protecting group is converted to the compound of formula IX by treating the compound of formula VIII with an aqueous mineral acid or catalytic hydrogenation. Any conventional aqueous mineral acid such as a hydrohalic acid can be utilized in carrying out this reaction to remove protecting groups which are hydrolyzed by conventional acid hydrolysis. On the other hand where R_3 is an amino protecting group removable by hydrogenation, any conventional method of catalytic hydrogenation can be utilized to remove this protecting group and convert the compound of formula VIII to the compound of formula IX.

The compound of formula IX where R_1 and R_2 do not form oxo, can be used to produce the anionic complex of formula VI either as a free base or in the form of its acid addition salt. Any conventional acid can be used in forming these salts. Among these acids are hydrochloric acid, phosphoric acid, acetic acid, propionic acid, citric acid, tartaric acid, etc.

The compound of formula IX is converted to the anionic complex of formula VI by reacting the compound of formula IX with a conventional salt of a radioactive metal. In forming the radioactive metal complex of formula X, any conventional radioactive isotope of technetium can be utilized. Among the radioactive isotopes are included technetium-99m. The aforementioned radioactive metals exist with coordination number of five in the complex of formula VI.

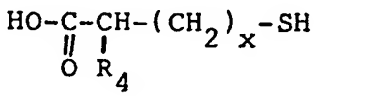
The compounds of formula II are known compounds and prepared from conventional protected amino acids of the formula:



XIII

wherein R and y are as above and R_{10} taken together with its attached oxygen atom form a hydrolyzable ester group such as a lower alkyl ester.

If in the compound of formula II, R_1 and R_2 are oxo, this compound is prepared by reacting the compound of formula XIII with a compound of the formula:

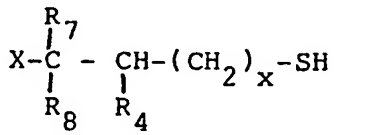


XIV

where x and R_4 are as above.

This reaction is carried out by amide condensation in the same manner as described in connection with the reaction of the compound of formulae II or II-A to produce a compound of formula I.

On the other hand where R_1 and R_2 in the compound of formula I are hydrogen or lower alkyl, the compound of formula II is produced by reacting the compound of formula XIII with a compound of the formula



XV

wherein R_4, R_7, R_8 and x are as above; and X is halogen.

The compound of formula XV is reacted with the compound of formula XIII utilizing any of the conventional techniques commonly employed in reacting primary amines with halides to produce secondary amines. In this manner, the compound of formula II wherein R_1 and R_2 are hydrogen or lower alkyl is produced. If it is desired to produce the compound of formula II in its free acid form, the reaction produced by reacting the compound of formula XIII with either the com-

pound of formulae XIV or XV is subjected to conventional ester hydrolysis. On the other hand, the thiol group in the compounds of formulae XIV or XV can, if desired, be protected with a conventional thiol protecting group prior to reaction with the compound of formula XIII. In this manner, the compound of formula II is produced wherein the thiol group is protected by a conventional thiol protecting group.

In forming the complex of radioactive technetium with the compound of formula IX the technetium complex of formula VI, the alkali metal salt of technetium-99m pertechnetate is reacted with the compound of formula IX in the presence of a reducing agent such as stannous chloride or sodium dithionite. The complex of formula VI can be prepared with the conventional salts of radioactive metals of technetium. Among these salts are the acetate, citrate and halide salts such as the chloride, bromide, fluoride and iodine salts of these radioactive metals. Among the technetium-99m pertechnetate salts are included the alkali metal salts such as the sodium salts or ammonium salts, or lower alkyl amine salts. The reaction of the compound of formula IX with the salt of the radioactive metal can be carried out in an aqueous medium at room temperature. The anionic complex of formula VI which has a charge of -1 can be formed in the aqueous medium in the form of a salt with a suitable cation such as ammonium cation, mono, di or tri-lower alkyl amine cation, etc. Any conventional salt of the anionic complex of formula VI with a pharmaceutically acceptable cation can be used in accordance with this invention. If it is desired to precipitate the anionic complex of formula VI, a salt is formed with a heavy cation such as tetraphenyl arsinate. Any conventional method of salt formation can be utilized to produce the chelate of formula VI as a salt. It is through the use of a precipitate with a heavy cation that this chelate can be characterized by structure.

In carrying out the reaction of the compound of formula IX with the salts of the radioactive metal to form the anionic complex of formula VI, the thiol protecting group is cleaved. Therefore, this reaction not only introduces the radioactive metal into the compound of formula VI but also cleaves the thiol protecting group. All of the aforementioned thiol protecting groups are cleaved by a reaction of salts of radioactive metals in accordance with this invention.

In forming the complex of formula VI, the radioactive material can have any suitable amount of radioactivity. In forming the radioactive anionic complexes of formula VI, it is generally preferred to form radioactive complexes in solutions containing radioactive concentrations of from about 0.01 mCi to 100 mCi per ml.

The complex of formula VI can be used for visualizing the organs such as the kidney for diagnosing disorders in these organs. In accordance with this invention, the anionic complex of formula VI either as a anionic complex or as a salt with a pharmaceutically acceptable cation are administered in a single unit injectable dose. Any of the common carriers such as sterile saline solution, plasma, etc., can be utilized for preparing the injectable solution to diagnostically image various organs in accordance with this invention. Generally, the unit dose to be administered has a radioactivity of about 0.01 mCi to about 100 mCi, preferably 1 mCi to 20 mCi. The solution to be injected to unit dosage is from about 0.01 ml to about 1 ml. After intravenous administration, imaging of the organ in vivo can take place in a matter of a few minutes. However, imaging can take place, if desired, in hours or even longer, after injecting into patients. In most instances, a sufficient amount of the administered dose will accumulate in the area to be imaged within about 0.1 of an hour to permit the taking of scintiphotos. Any conventional method of imaging for diagnostic purposes can be utilized in accordance

with this invention.

The complexes of formula VI may be administered intravenously in any conventional medium for intravenous injection such as an aqueous saline medium, or in blood plasma medium. Such medium may also contain conventional pharmaceutical adjunct materials such as, for example, pharmaceutically acceptable salts to adjust the osmotic pressure, buffers, preservatives and the like. Among the preferred mediums are normal saline and plasma.

The following examples are illustrative but not limitative of the invention. The percent (%) yields in the following examples are given based upon the mols of starting material.

Example 1

N-(t-Butyloxycarbonyl)cysteamine-N-acetic acid

Cysteamine-N-acetic acid hydrochloride, 5.16 g (30 mmoles), is dissolved in 100 ml of deioniued water and 10.0 ml (72 mmoles) of freshly distilled Et_3N is added. N-t-butyloxycarbonyloxyimino-2-phenylacetonitrile, 7.5 g (30 mmoles), is dissolved in 50 ml of dioxane and added in one portion to the cysteamine solution. The reaction mixture is stirred at room temperature overnight. The solution is reduced to 50 ml by rotary evaporation and poured into 200 ml EtOAc. The EtOAc phase is extracted with 3 portions of 100 ml saturated $NaHCO_3$. The $NaHCO_3$ portions are combined and the pH is lowered to 3.5 by addition of solid citric acid. The yellow product is extracted into 250 ml of EtOAc. The EtOAc is dried over anhydrous Na_2SO_4 and removed by rotary evaporation. After drying under vacuum overnight, 4.25 g (60% yield) of the yellow glassy compound, N-(t-butyloxycarbonyl)cysteamine-N-acetic acid, is obtained.

Example 2

N-(t-butyloxycarbonyl)-N-(2-mercaptoethyl)glycyl homocysteine thiolactone

a) Mixed Anhydride Method

N-(t-butyloxycarbonyl)cysteamine-N-acetic acid, 1.50 g (6.4 mmoles), is dissolved in 50 ml CH_2Cl_2 by addition of 1.0 ml (7.2 mmoles) of freshly distilled Et_3N. This solution is cooled to 0°C on an ice bath under an argon atmosphere and 0.85 ml (6.5 mmoles) of isobutylchloroformate is added dropwise over a period of five minutes. The solution turns orange-red after 15 minutes of stirring. A solution of 1.16 g (7.5 mmoles) of homocysteine thiolactone hydrochloride dissolved in 100 ml of CH_2Cl_2 by addition of 2.2 ml (15.8 mmoles) of Et_3N is added dropwise over 15 minutes. The solution is stirred at 0°C for 4 hours and left to stir

at room temperature overnight. The CH_2Cl_2 solution is extracted with 2 x 100 ml of 5% by weight aqueous citric acid, 2 x 100 ml of saturated $NaHCO_3$, and 100 ml saturated NaCl. The CH_2Cl_2 is dried over anhydrous Na_2SO_4 and removed by rotary evaporation. After drying under vacuum overnight, 1.5 g (75% yield) of a white glass is obtained. TLC in 5% by volume methanol-95% by volume chloroform showed the major spot at Rf = 0.4. The white glass product was purified by silica gel chromatography to yield 0.9 g (45% yield) of N-(t-butyloxycarbonyl), N-(2-mercaptoethyl)-glycyl homocysteine thiolactone as a white crystalline solid.

b) Carbodiimide Method

N-(t-butyloxycarbonyl)cysteamine-N-acetic acid, 1.41 g (6 mmoles); 1-hydroxybenzotriazole, 1.22 g (9 mmoles); homocysteine thiolactone hydrochloride, 1.0 g (6.5 mmoles); and 2.5 ml (14 mmoles) of freshly distilled Et_3N are dissolved in 50 ml of CH_2Cl_2. The solution is cooled to 0°C and a solution of 1.24 g (6 mmoles) of N,N'-dicyclohexylcarbodiimide in 10 ml CH_2Cl_2 is added in one portion. The solution is stirred on ice for 4 hours and left to stir at room temperature overnight. The N,N'-dicyclohexylurea is filtered and the CH_2Cl_2 solution is extracted with 2 x 100 ml 5% by weight aqueous citric acid, 2 x 100 ml saturated $NaHCO_3$, and 100 ml saturated NaCl. The CH_2Cl_2 is dried over anhydrous Na_2SO_4 and removed by rotary evaporation. After drying under vacuum, 2.1 g of a light pink crystalline product is obtained.

TLC in 5% methanol-95% chloroform showed the major spot at Rf = 0.4. The product was purified by silica gel chromatography to yield 0.75 g (37.5% yield) of the white crystalline solid N-(t-butylcarbonyl), N-(2-mercaptoethyl)-glycyl homocysteine thiolacetone.

Example 3

N-(t-butyloxycarbonyl), N-(2-mercaptoethyl)glycyl N'-methyl-homocysteinamide

N-(t-butyloxycarbonyl), N-(2-mercaptoethyl)glycyl homocysteine thiolacetone, 1.0 g (3 mmoles), is dissolved in 25 ml THF and cooled to 0°C. The solution is saturated with methylamine by bubbing methylamine gas through for 10 minutes. The reaction is stirred for 30 minutes and the solvent removed by rotary evaporation. After drying overnight under vacuum, 1.09 g (100% yield) of N-(t-butyloxycarbonyl), N-(2-mercaptoethyl)glycyl N'-methylhomocysteinamide is obtained as a white glass.

Example 4

N-(2-mercaptoethyl)glycyl N'-methylhomocysteinamide hydrochloride

N-(t-butyloxycarbonyl), N-(2-mercaptoethyl)glycyl N'-methylhomocysteinamide, 1.09 g (3 mmoles), is dissolved in 25 ml THF and cooled to 0°C. HCl gas is bubbled through the solution. After one minute, a white precipitate begins to form. The bubbling is continued for 15 minutes and the mixture is stirred for 15 more minutes. The white precipitate is filtered and washed with THF being careful not to dry by air suction. The still wet white product is vacuum dried overnight to yield 550 mg (60% yield) of white, crystalline N-(2-mercaptoethyl)glycyl N'-methylhomocysteinamide hydrochloride.

Example 5

Tetraphenylarsonium salt of [2-[[1-(2-mercaptoethyl)-2-(methylamino)-2-oxoethyl]amino]-N-(2-mercaptoethyl)glycinato-N,N',S,S']oxotechnetate-99

$NH_4{}^{99}TcO_4$, 50 mg (0.28 mmoles), and N-(2-mercaptoethyl)glycyl N'-methylhomocysteinamide hydrochloride, 160 mg (0.53 mmoles), were dissolved in 2 ml of 1:1 parts by volume

EtOH and 2N NaOH mixture. A solution of 50 mg (0.29 mmoles) of $Na_2S_2O_4$ in 1.0 ml 2N NaOH was added dropwise to the stirred solution turning it from clear to deep orange. Electrophoresis of the orange solution in tris-bartital-sodium barbital buffer at pH 8.8 run at 600V for 45 minutes on paper showed that the orange complex migrated 7.2 cm toward the anode. Electrophoresis indicated a small amount ($< 1\%$) of black TcO_2 that stayed at the spotting point but showed no TcO_4^- at its characteristic 12.5 cm migration. The orange solution was filtered to remove the black, insoluble TcO_2 and was added to a solution of 150 mg (0.36 mmoles) of $Ph_4AsCl.H_2O$ in 2.0 ml of water. An orange oil separated and was dissolved by adding about 2 ml of methanol. This solution was left to stand in an open beaker for several days. After one week orange crystals formed at the bottom of the beaker and the once dark orange solution was now faintly yellow. The crystals were filtered, washed with a small amount of cold water followed by Et_2O, and dried by air suction to yield 180 mg (86% yield) of tetraphenylarsonium salt of [2-[[1-(2-mercaptoethyl)-2-(methylamino)-2-oxoethyl]amino]-N-(2-mercaptoethyl)glycinato-N,N',S,S']-oxotechnetate-99 as bright orange crystals. Dark orange plate-like crystals suitable for x-ray structure determination were grown by slow evaporation of an ethanol-chloroform solution of the product.

Example 6

[2-[[1-(2-mercaptoethyl)-2-(methylamino)-2-oxoethyl]amino]-N-(2-mercaptoethyl)glycinato-N,N',S,S']-oxotechnetate-99m

1. Dithionite Reduction at pH 13.3

N-(2-mercaptoethyl)glycyl N'-methylhomocysteinamide hydrochloride, 5 mg (0.17 mmoles), is dissolved in 1.0 ml absolute EtOH and 1.0 ml 1.0N NaOH. A 1.0 ml generator eluant of $^{99m}TcO_4^-$ (5 to 50 mCi) in saline is added. Then 0.5 ml of a dithionite solution, prepared by dissolving 100 mg $Na_2S_2O_4$ per ml of 1.0N NaOH, is added. After 15-30

minutes, the [2-[[1-(2-mercaptoethyl)-2-(methylamino)-2-oxoethyl]amino]-N-(2-mercaptoethyl)glycinato-N,N',S,S']-oxotechnetate-99m is prepared [^{99m}-Tc-MGHA complex]. This solution of the ^{99m}Tc-MGHA complex is buffered by addition of 1.0 ml of 1N HCl and 4.0 ml of 0.1M NaH_2PO_4 pH 4.5 buffer. The labelling of this complex is determined by electrophoresis in tris-barbital-sodium barbital buffer at pH 8.8 and was run at 600V for 45 minutes on paper. The ^{99m} Tc-MGHA complex migrates 7.2 cm toward the anode under these electrophoretic conditions. The possible impurities, $^{99m}TcO_2$ and $^{99m}TcO_4^-$, are easily distinguished from the ^{99m}Tc-MGHA complex by this electrophoresis method because the unreduced ^{99m} TcO_4^- migrates 12.5 cm toward the anode while ^{99m} TcO_2 remains at the origin.

2. Stannous Reduction at pH 6.5

N-(2-mercaptoethyl)glycyl N'-methylhomocysteinamide hydrochloride, 5 mg (0.17 mmoles), is dissolved in 1.0 ml EtOH and 1.0 ml 0.1M sodium acetate at pH 5.5. A 1.0 ml generator eluant of ^{99m} TcO_4^- (5-50 mCi) in saline is added. Then 0.2 ml of stannous solution, prepared by dissolving 2.0 mg $SnCl_2.2H_2O$ per ml of ethanol, is added to produce [2-[[1-(2-mercaptoethyl)-2-(methylamino)-2-oxoethyl]amino]-N-(2-mercaptoethyl)glycinato-N,N',S,S']oxotechnetate-99m. After 15-30 minutes, the labeling efficiency is determined by electrophoresis as described under the dithionite reduction method above with the same results.

Example 7

N-[2-(S-acetamidomethyl)mercaptopropionyl]glycine

2-Mercaptopropionylglycine, 20.0 g (122.6 mmoles), and N-hydroxymethyl-acetamide, 12.0 g (134.8 mmoles), are dissolved in 200 ml of deionized water. The solution is cooled on an ice bath and 100 ml of conc. HCl is added in one portion. The mixture is stirred on ice for one hour and at room temperature overnight. A white precipitate begins to form within 1-2 hours.

The reaction mixture is cooled on ice for 4 hours. The white precipitate is filtered, washed with a few mls of ice-cold water, then 2 x 200 ml Et_2O. The product is dried by air suction for one hour and uncer vacuum overnight to yield 14.4 g (50.2% yield) of the white, crystalline product N-[2-(S-acetamidomethyl)mercaptopropionyl]-glycine.

Example 8

N-[2-(S-benzamidomethyl)mercaptopropionyl]glycine

2-Mercaptopropionylglycine, 5.0 g (30.6 mmoles), and N-hydroxymethylbenzamide, 5.0 g (33.1 mmoles), are dissolved in 100 ml of deionized water. The solution is cooled on an ice bath and 50 ml of conc. aqueous HCl is added in one portion. The mixture is stirred on ice for one hour and at room temperature overnight. A white precipitate begins to form within thirty minutes. After this period the reaction mixture is cooled on ice for 4 hours. The white precipitate is filtered, washed with ice-cold water, then 2 x 200 ml Et_2O. The product is dried by air suction for an hour and under vacuum overnight to yield 8.4 g (94.4% yield) of the white, crystalline product N-[2-(S-benzamidomethyl)mercaptopropionyl]glycine.

Example 9

N-[2-(S-acetamidomethyl)mercaptopropionyl]glycyl homocysteine thiolactone

1. Carbodiimide Method

N-[2-(S-acetamidomethyl)mercaptopropionyl]glycine, 2.35 g (10 mmoles), homocysteine thiolactone hydrochloride, 1.55 g (10 mmoles), 1-hydroxybenzotriazole, 2.1 g (15 mmoles), and 4.5 ml of Et_3N are dissolved in 150 ml CH_2Cl_2. A solution of 2.0 g (9.7 mmoles) of dicyclohexylcarbodiimide in 50 ml CH_2Cl_2 is then added in one portion and the mixture is stirred overnight.

The reaction mixture is filtered to remove the white precipitate which is dicyclohexylurea. The solvent is removed by rotary evaporation. After this, the residue is vacuum dried overnight, and 7.8 g of crude material remains. The crude product is dissolved in 5 ml of MeOH: $CHCl_3$ (5/95 parts by volume) and loaded on a silica gel column of 8 cm diameter and 10 cm height requiring 250 g of silica gel. The column is eluted with MeOH:$CHCl_3$ (5/95 parts by weight) and 5 ml fractions are collected. The product at R_f = 0.3 on TLC in MeOH:$CHCl_3$ (10/90 parts of volume) elutes in fractions 110-190. These fractions are combined and the solvent is removed by rotary evaporation. After removal of solvent, the residue is dried under vacuum overnight, 2.0 g (60.6% yield) of N-[2-S-acetamidomethyl)mercaptopropionyl]glycyl homocysteine thiolactone as a white glass is obtained.

2. Mixed Anhydride Method

N-[2-(S-acetamidomethyl)mercaptopropionyl]glycine, 7.05 g (30 mmoles), is dissolved in 150 ml CH_2Cl_2 by addition of 7.5 ml (54 mmoles) of Et_3N. The solution is cooled to -15°C on a dry ice-acetone bath. Isobutylchloroformate, 3.9 ml (30 mmoles), is added dropwise over a 5 minute period. The temperature is maintained at -15°C throughout the addition. After the addition of isobutylchloroformate is completed, the reaction is stirred from 3-5 minutes. A solution of 4.65 g (30 mmoles) of homocysteine thiolacetone hydrochloride and 4.2 ml (30 mmoles) of Et_3N in 100 ml of CH_2Cl_2 is added dropwise over a period of 15-30 minutes at a rate that maintains the temperature at -15°C. The solution is then stirred at -15°C for one hour and at room temperature overnight.

The white precipitate that forms is filtered, washed with a small amount of CH_2Cl_2, dried by air suction for one hour and under vacuum overnight. This yields 3.2 g (32% by weight yield) of N-[2-(S-acetamidomethyl)mercaptopropionyl]-glycyl homocysteine thiolactone as a fine, white powder.

TLC on silica gel in MeOH:$CHCl_3$ (10/90 parts by volume) shows a single spot at R_f = 0.3.

A second crop of N-[2-(S-acetamidomethyl)mercaptopropionyl]glycyl homocysteine thiolactone 700 mg (40% overall yield) of TLC-pure product was obtained by evaporating the filtrate, dissolving the residue in a minimum of MeOH, and letting the solution stand for two days.

Example 10

N-[2-(S-benzamidomethyl)mercaptopropionyl]glycyl homocysteine thiolacetone

1. Carbodiimide Method

N-[2-(S-benzamidomethyl)mercaptopropionyl]glycine, 2.96 g (10 mmoles), homocysteine thiolactone hydrochloride, 1.55 g (10 mmoles), 1-hydroxybenzotriazole, 2.1 g (15 mmoles), and 4.5 ml Et_3N is dissolved in 100 ml CH_2Cl_2. A solution of 2.0 g (9.7 mmoles) of dicyclohexylcarbodiimide in 50 ml CH_2Cl_2 is then added in one portion and the mixture is stirred overnight.

The dicyclohexylurea that precipitates is filtered and the CH_2Cl_2 solution is extracted with 2 x 200 ml 10% by weight aqueous HCl, 2 x 200 ml saturated $NaHCO_3$, and 200 ml saturated NaCl. The CH_2Cl_2 layer is dried over anhydrous Na_2SO_4 and removed by rotary evaporation. The residue is dried under vacuum overnight to yield 2.0 g of a white glass.

The crude product is dissolved in 3.0 ml of MeOH:$CHCl_3$ (5/95 parts by volume) and loaded on a silica gel column of 4.5 cm diameter and 12.5 cm height requiring 100 g of silica gel. The column is eluted with MeOH:$CHCl_3$ (5/95 parts by volume) and 4 ml fractions are collected. The product at R_f = 0.4 on TLC in MeOH:$CHCl_3$ (10/90 parts by volume) elutes in fractions 51-70. The fractions are com-

bined and the solvent is removed by rotary evaporation. After the residue is dried under vacuum overnight, 1.0 g (26.3% yield) of the white glass N-[2-(S-benzamidomethyl)-mercaptopropionyl]glycyl homocysteine thiolactone is obtained.

2. Mixed Anhydride Method

N-[2-(S-benzamidomethyl)mercaptopropionyl]glycine, 2.96 g (10 mmoles) is dissolved in 100 ml CH_2Cl_2 by addition of 3.0 ml (22 mmoles) of Et_3N. The solution is cooled to -15°C on a dry ice-acetone bath. Isobutylchloroformate, 1.3 ml (10 mmoles), is added dropwise over a 5 minute period. The temperature is maintained at -15°C throughout the addition. After the addition of isobutylchloroformate is completed, the reaction is stirred for 3-5 minutes. A solution of 1.55 g (10 mmoles) of homocysteine thiolactone hydrochloride and 1.4 ml (10 mmoles) of Et_3N in 50 ml CH_2Cl_2 is added dropwise over a period of 15-30 minutes. The temperature is maintained at -15°C throughout the addition. The solution is then stirred at -15°C for one hour and at room temperature overnight.

The CH_2Cl_2 solution is washed with 2 x 200 ml 10% by volume aqueous HCl, 2 x 200 ml saturated $NaHCO_3$, and 200 ml saturated NaCl. The CH_2Cl_2 layer is dried over anhydrous Na_2SO_4 and removed by rotary evaporation. The residue is dried under vacuum overnight to yield 2.7 g (71.0% yield) of N-[2-(S-benzamidomethyl)mercaptopropionyl]glycyl homocysteine thiolacetone as a white glass.

Example 11

N-[2-(S-acetamidomethyl)mercaptopropionyl]glycyl N-methyl-homocysteinamide

N-[2-(S-acetamidomethyl)mercaptopropionyl]glycyl homocysteine thiolactone, 1.0 g (3 mmoles), is suspended in 200 ml THF. Methylamine gas is bubbled vigorously through the suspension. Most of the white starting material goes

into solution in 5-10 minutes. The gas is bubbled for 15 minutes more at as low rate. TLC on silica gel in MeOH:$CHCl_3$ (15/85 parts by volume) shows that the reaction is complete. The excess CH_3NH_2 and also THF is removed by rotary evaporation. After the residue is dried under vacuum overnight, 1.1 g (100% yield) of the white glass N-[2-(S-acetamidomethyl)mercaptopropionyl]glycyl N-methyl-homocysteinamide is obtained.

Example 12

By the procedure of Example 11 N-[2-(S-benzamido-methyl)mercaptopropionyl]glycyl homocysteine thiolactone, converted to N-[2-(S-benzamidomethyl)mercaptopropionyl]-glycyl N-methylhomocysteinamide.

Example 13

Tetraphenylarsonium salt of [1-[[1-(2-mercaptoethyl)-2-(methylamino)-2-oxoethyl]amino]-N-(2-mercapto-1-1-oxo-propyl)glycinato-N,N',S,S']oxotechnetate-99

$NH_4{}^{99}TcO_4$, 50.3 mg (0.28 mmoles), and 200.1 mg (0.55 mmoles) of N-[2-(S-acetamidomethyl)mercaptopropionyl]-glycyl N-methylhomocysteinamide, were dissolved in 5.0 ml of 1:1 parts of volume EtOH:2NNaOH. A solution of 60 mg (0.35 mmoles) $Na_2S_2O_4$ in 1.0 ml 2NNaOH was added dropwise. A bright yellow solution resulted and the solution turned dark orange after sitting for one hour. Electrophoresis of the orange solution was then run in barbital buffer at pH 8.8 at 600V for 45 minutes on paper. The orange complex migrated 6.9 cm toward the anode indicating an anionic complex. No TcO_2 or TcO_4 impurties, which come at 0.0 and 12.5 cm, respectively, were observed. A solution of 250 mg (0.60 mmoles) of $Ph_4AsCl.H_2O$ in 2.0 ml H_2O was added to the orange solution and it was left undisturbed overnight. The next day orange plates had formed at the bottom of the vial and the solution had only a light yellow color. The product was filtered, washed with water, and dried by air

suction overnight to yield 158.8 mg (72.2% yield) of the tetraphenylarsonium salt of [1-[[1-(2-mercaptoethyl)-2-(methylamino)-2-oxoethyl]amino]-N-(2-mercapto-1,1-oxopropyl)glycinato-N,N',S,S']oxotechnetate-99 as golden-orange plates.

Example 14

1. Dithionite Reduction at pH 13

N-[2-(S-acetamidomethyl)mercaptopropionyl]glycyl N-methylhomocysteinamide, 20 mg (0.55 mmoles), is dissolved in 2.0 ml 1:1 parts by volume of EtOH and 1NNaOH. A 0.5 ml generator eluant of $^{99m}TcO_4^-$ (5 mCi) is added. Then 0.5 ml of a dithionite solution, prepared by dissolving 100 mg $Na_2S_2O_4$ per ml of 1NNaOH, is added. The vial is left to stand for 30 minutes. Electrophoresis shows an anionic complex which is [1-[[1-(2-mercaptoethyl)-2-(methylamino)-2-oxoethyl]amino]-N-(2-mercapto-1,1-oxopropyl)glycinato-N,N',S,S']oxotechnetate-99m and also shows the absence of the possible impurities $^{99m}TcO_2$ and $^{99m}TcO_4^-$.

2. Stannous Reduction at pH 6.5

N-[2-(S-acetamidomethyl)mercaptopropionyl]glycyl homocysteinamide, 20 mg (0.55 mmoles), is dissolved in 1.0 ml 0.1M sodium acetate pH 4.5 buffer and 1.0 ml EtOH. A 0.5 ml generator eluant of $^{99m}TcO_4^-$ (5 mCi) is added. Then 0.2 ml of a stannous solution, prepared by dissolving 2.0 mg $SnCl_2.2H_2O$ per ml EtOH, is added. The vial is left to stand for 30 minutes. Electrophoresis shows an anionic complex which is [1-[[1-(1-mercaptoethyl)-2-(methylamino)-2-oxoethyl]amino]-N-(2-mercapto-1,1-oxopropyl)glycinato-N,N',S,S']oxotechnetate-99m.

Example 15

By both the dithionite reduction at pH 13 procedure in Example 14 and the stannous reduction at pH 6.5 in Example 14, N-[2-(S-benzamidomethyl)mercaptopropionyl]-

glycyl N-methylhomocysteinamide is converted to [1-[[1-(2-mercaptoethyl)-2-(methylamino]-2-oxoethyl]amino]-N-[2-mercapto-1,1-oxopropyl)glycinato-N,N',S,S']oxotechnetate-99m.

Example 16

The ^{99m}Tc complex used for the animal studies was prepared in Example 6 following both methods of reduction, stannous chloride or dithionite. The ^{99m}Tc-MGHA complexes labeled by either reduction method were tested in animals. The mass concentrations of the ^{99m}Tc complex administered to test animals were approximately 0.04 mg/kg body weight for rabbits and 13.0 mg/kg body weight for mice. The radioactivity administered to rabbits and mice were about 0.5 mCi and 1.5 mCi respectively. The pH of the injectant solution was approximately 5 to 6.

Nonanesthetized and nonfasted male rabbits (New Zealand White) weighing 3.0-3.5 kg were restrained in dorsal recumbancy approximately 2-3 cm from the face of the gamma camera. After intravenous administration of 0.1 ml of the ^{99m}Tc-labeled complex solution in the marginal ear vein, anterior images were stored by a dedicated computer during injection, and 5, 10, 15, 20 and 30 minutes following administration. Relative counts per minute (cpm) versus time curves (uncorrected for ^{99m}Tc decay) were determined from quantification of regional areas of interest (RAI) obtained from computer displayed images for ^{99m}Tc complex.

Nonfasted male mice (ICR strain) weighing 20-24 grams were administered in the tail vein with 0.2 ml of a ^{99m}Tc-complex solution. At 15, 30, 60 minutes post administration groups of four animals were sacrificed by cervical dislocation and selected organs removed for ^{99m}Tc assay in a gross ionization counting chamber. The percentage of the injected dose corrected for ^{99m}Tc decay was calculated for the various organs at each sacrifice time.

The RAIs obtained from the gamma camera images for animals administered with ^{99}Tc-complex ($SnCl_2$ reduction method) and ^{99m}Tc-complex (dithionite reduction method) showed significant count densities in regions where the right and left kidneys were visualized. No other apparent anatomical structures were clearly delineated. The organ distribution of ^{99m}Tc activity in mice after administration of ^{99m}Tc-complex indicated fast clearance out of the body within the first 15 minutes. Subsequent sacrifice times greater than 15 minutes post injection showed little kidney uptake. Although kidney uptake of activity was declining at the various time intervals examined, the total remaining activity (% injected dose) in each test animal was approximately one-half of the injected dose at 30 minutes which indicated a rapid excretion of activity from the body. This indirect evidence suggests possible kidney clearance of ^{99m}Tc in test animals administered ^{99m}Tc-complex.

Example 17

The ^{99m}Tc-complex prepared via a dithionite reduction of Example 6 was administered to male albino rabbits weighing 3.0-3.5 kg. The drug was injected into the marginal ear vein. The mass of drug administered was equivalent to ~0.04 mg/kg while the volume injected was 0.1 ml. The administered dose was ~0.5 mCi. Each test animal was restrained in dorsal recumbancy approximately 2-3 cm from the face of the gamma camera. Immediately after injection, 100-500 k counts were obtained from the gamma camera and stored on floppy disc. The computer controlled the maximum number of counts that could be stored per image. Sequential images were obtained and stored on disc at 5, 10, 15, 20 and 30 minutes following administration.

After all images were stored, quantitative image analysis was utilized to obtain count densities in regional

areas of interest (RAI). The RAIs were delineated as distinct anatomical structures. Three distinct areas were observed. The left and right kidneys and the bladder were clearly defined.

Example 18

A ^{99m}Tc-complex prepared via a stannous chloride reduction of Example 6 was administered to male albino rabbits weighing 3.0-3.5 kg. The route of administration and the methods for evaluation of this test drug are described in Example 17.

Example 19

A ^{99m}Tc-MGHA complex prepared via a dithionite reduction of $^{99m}TcO_4^-$ of Example 6 was administered to female albino mice weighing 20-24 gm. The test drug was administered through the lateral tail vein at a mass dose of approximately 13.0 mg/kg in a volume of 0.2 ml. The corresponding radioactive dose administered to each animal was 1.5 mCi. After injection, groups of four animals were sacrificed (cervical dislocation) at 15, 30 and 60 minutes. The liver, kidney, lung, heart, stomach and intestines (small and large) were removed and assyed for ^{99m} Tc activity in a gross ionization counting chamber. Also, the tails and remaining carcasses were individually assayed for ^{99m}Tc activity.

Knowing the amount of ^{99m}Tc activity administered (assaying for ^{99m}Tc in the syrine before and after injection) and the amount assayed in the various organs, the percentage of injected dose (% ID) was calculated as:

$$\% \text{ ID} = \frac{\text{Ci of activity in organ}}{\text{Ci of activity injected}} \times 100\%$$

All activities (Ci) were initially corrected for ^{99m}Tc decay back to a fixed starting time using the standard first order differential equation for decay correction (see below):

$$N_1 = N_0 e^{-(.693/T_{1/2})t}$$

where N_0 and N_1 are initial and final activities (Ci) respectively. The half-life ($T_{1/2}$) for ^{99m}Tc decay is 6.02 hr. The value of t is the elapsed time (hrs.) for the correction.

The results of ^{99m}Tc uptake in various organs and tissues in mice administered ^{99m}Tc-MGHA labeled via a dithionite reduction is given in Table 1.

Example 20

A ^{99m} Tc-MGHA complex prepared via a stannous chloride reduction of $^{99m}TcO_4^-$ of Example 6 was administered to female albino mice weighing 20-24 gm. The same methods and procedures given in Example 19 were used to calculate the % ID for each organ. The results are given in Table 2.

Table 1

Organ Uptake of ^{99m}Tc in Mice After Intravenous Administration of ^{99m}Tc-MGHA (Dithionite Reduction)

	(% Injected Dose) Time After Injection		
Organ	15 min.	30 min.	60 min.
Liver	11.3±1.0[1]	7.6±0.9	10.2±1.6
Spleen	N.D.[2]	N.D.	N.D.
Kidney	3.1±0.6	3.4±0.5	3.9±0.6
Heart	N.D.	N.D.	N.D.
Lung	0.9±0.2	0.3±0.1	0.5±0.3
Stomach	0.3±0.01	-0.1±0.1	0.8±0.9
Carcass	21.9±2.9	13.4±5.3	18.3±2.5
Intestines	16.8±3.0	20.5±2.0	16.9±1.4
Tail	1.7±1.0	0.3±0.1	1.1±0.4

[1] Average of four animals ± standard deviation.

[2] N.D. = no detectable activity.

Table 2

Organ Uptake of ^{99m}Tc in Mice After Intravenous Administration of ^{99m}Tc-MGHA ($SnCl_2$ Reduction)

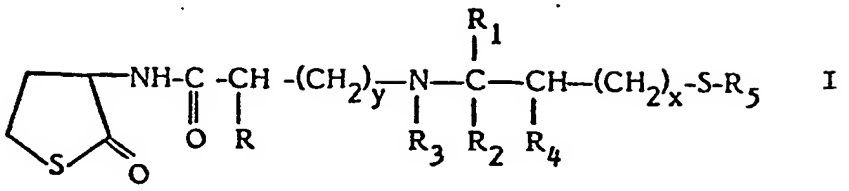
	(% Injected Dose) Time After Injection		
Organ	15 min.	30 min.	60 min.
Liver	13.3±5.4[1]	6.1±0.9	7.7±2.0
Spleen	N.D.[2]	N.D.	N.D.
Kidney	3.3±0.9	1.6±0.4	2.3±0.8
Heart	N.D.	N.D.	N.D.
Lung	0.9±0.4	0.2±0.2	0.6±0.3
Stomach	0.3±0.4	0.6±0.9	0.1±0.2
Carcass	17.4±2.8	5.7±2.9	12.4±5.1
Intestines	28.7±6.1	27.3±7.0	29.3±10.4
Tail	1.4±0.7	0.14±0.2	0.7±0.3

[1] Average of four animals ± standard deviation.

[2] N.D. = No detectable activity.

CLAIMS

1. A compound of the formula



I

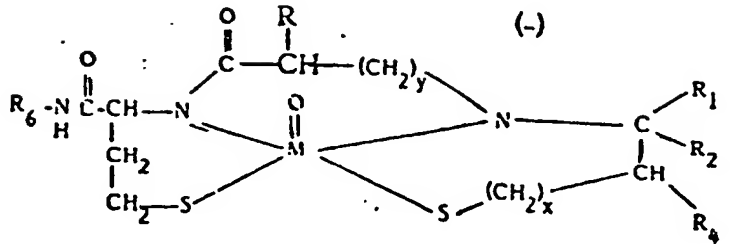
wherein R is hydrogen or lower alkyl; R_1 and R_2 are individually hydrogen or lower alkyl or taken together form oxo; R_3 is an amino protecting group where R_1 and R_2 are individually hydrogen or lower alkyl; R_3 is hydrogen when R_1 and R_2 taken together form oxo; R_4 is hydrogen or lower alkyl, R_5 is hydrogen or a thiol protecting group; and x and y are integers from 0 to 2.

2. The compound of claim 1 wherein said compound is N-(t-butyloxycarbonyl), N-(2mercaptoethyl)glycyl homocysteine thiolactone.

3. The compound of claim 1 wherein said compound is N-[2-(S-acetamidomethyl)mercaptopropionyl]glycyl homocysteine thiolactone.

4. The compound of claim 1 wherein said compound is N-[2-(S-benzamidomethyl)mercaptopropionyl]glycyl homocysteine thiolactone.

5. An anionic chelate of the formula



VI

wherein R_6 is lower alkyl; R_1 and R_2 are individually hydrogen or lower alkyl or taken together form oxo; M is a radioactive metal; R_4 is hydrogen or lower alkyl; x and y are integers from 0 to 2 or a salt of said anionic chelate.

6. The chelate of claim 5 wherein M is selected from the group consisting of radioactive technetium.

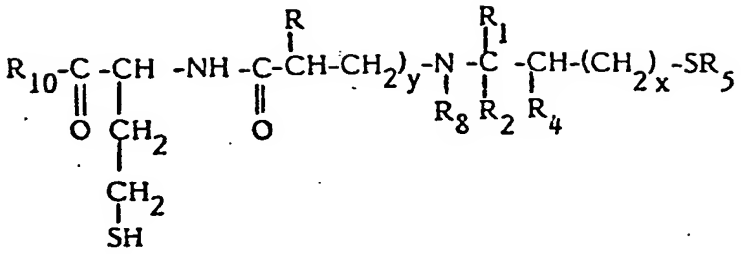
7. The chelate of claim 5 wherein M is technetium-99m.

8. The chelate of claim 7 wherein said chelate is [2-[[1-(2-mercaptoethyl)-2-(methylamino)-2-oxoethyl]amino]-N-(2-mercaptoethyl)glycinato N,N',S,S']oxotechnetate-99m.

9. The chelate of claim 8 wherein said chelate is in the form of its alkali metal salt, ammonium salt or amine salts.

10. The chelate of claim 5 wherein said chelate is [1-[[1-(2-mercaptoethyl)-2-(methylamino)-2-oxoethyl]amino]-N-(2-mercapto-1,1-oxopropyl)glycinato-N,N',S,S']oxo-technetate-99m.

11. A compound of the formula



wherein R_{10} is -OH or R_6-NH-; R_6 is lower alkyl R_1 and R_2 are individually hydrogen or lower alkyl or taken together form oxo; R_8 is hydrogen or an amino protecting group; R_4 is hydrogen or lower alkyl; R_5 is hydrogen or a thiol protecting group and x and y are integers from 0 to 2 with the proviso that when R_1 and R_2 form oxo; R_8 is hydrogen or salts thereof.

12. The compound of claim 11 wherein R_5 is hydrogen.

13. The compound of claim 11 wherein said compound is N-(t-butyloxycarbonyl), N-(2-mercaptoethyl)glycyl N'-methyl-homocysteinamide.

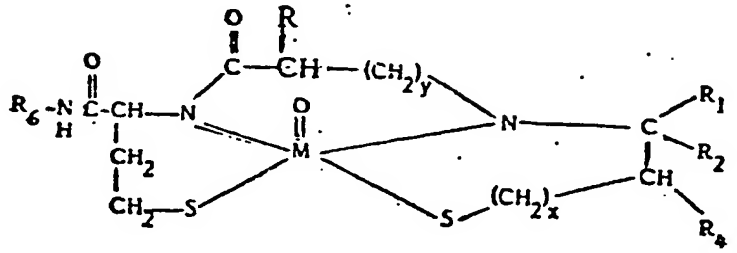
14. The compound of claim 12 wherein said compound is N-(2-mercaptoethyl)glycyl N'-methylhomocysteinamide.

15. The compound of claim 11 wherein R is a thiol protecting group.

16. The compound of claim 14 wherein said compound is N-[2-(S-acetamidomethyl)mercaptopropionyl]glycyl N-methyl-homocysteinamide.

17. The compound of claim 13 wherein said compound is N-[2-(S-benzylamidomethyl)mercaptopropionyl]glycyl N-methyl-homocysteinamide.

18. A composition suitable for intravenous injection comprising an anionic chelate of the formula:



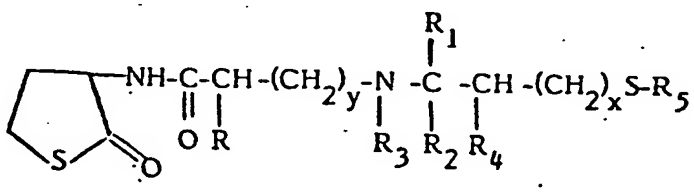
wherein R is hydrogen or lower alkyl; R_6 is lower alkyl; R_1 and R_2 are individually hydrogen or lower alkyl or taken together form oxo; M is a radioactive metal; R_4 is hydrogen or lower alkyl; x and y are integers from 0 to 2

or a salt of said anionic chelate said chelate or said salt being present in an amount sufficient to provide radioactivity of from 101 mCi to 100 mCi and a carrier suitable for intravenous injection.

19. The composition of claim 18 wherein said chelate is [2-[[1-(2-mercaptoethyl)-2-(methylamino)-2-oxoethyl]amino]-N-(2-mercaptoethyl)glycinato N,N',S,S']oxotechnetate-99m.

20. The composition of claim 19 wherein said chelate is [1-[[1-(2-mercaptoethyl)-2-(methylamino)-2-oxoethyl]amino]-N-(2-mercapto-1,1-oxopropyl)glycinato-N,N',S,S']-oxotechnetate-99m.

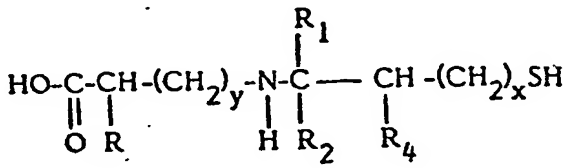
21. A process for the preparation of a compound of the formula



wherein R is hydrogen or lower alkyl; R_1 and R_2 are individually hydrogen or lower alkyl or taken together form oxo; R_3 is an amino protecting group where R_1 and R_2 are individually hydrogen or lower alkyl; R_3 is hydrogen when R_1 and R_2 taken together form oxo; R_4 is hydrogen or lower alkyl, R_5 is hydrogen or a thiol protecting group; and x and y are integers from 0 to 2,

which comprises

a) reacting a compound of formula

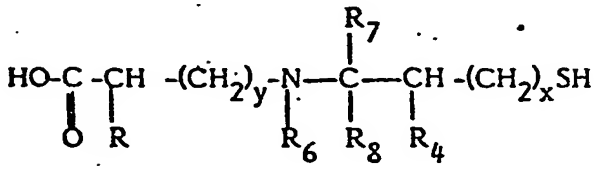


II

wherein R, R_1, R_2, R_3, R_4, x and y are as above,

or

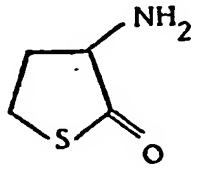
b) reacting a compound of formula



II-A

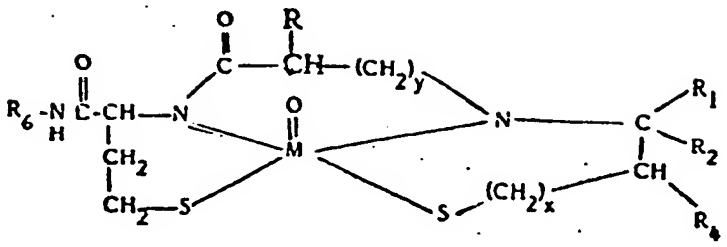
wherein x, y, R and R_4 are as above; R_6 is an amino protecting group; R_7 and R_8 are individually hydrogen or lower alkyl,

with a compound of formula



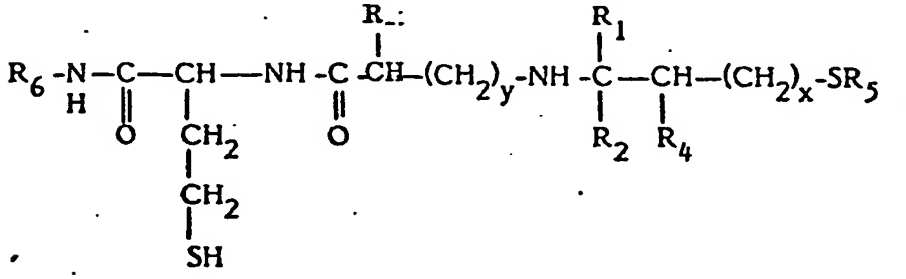
IV

22. A process for the preparation of an anionic chelate of the formula



VI

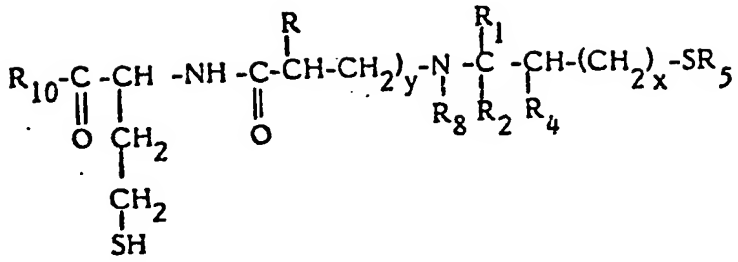
wherein R_6 is lower alkyl; R_1 and R_2 are individually hydrogen or lower alkyl or taken together form oxo; M is a radioactive metal; R_4 is hydrogen or lower alkyl; x and y are integers from 0 to 2 or a salt of said anionic chelate, which comprises reacting a compound of the formula



IX

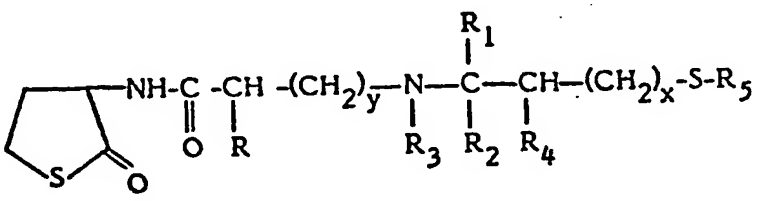
wherein R, R_1, R_2, x, y, R_3, R_4, R_5 and R_6 are as above, with the proviso that R_3 is an amino protecting group when R_1 and R_2 are individually hydrogen or lower alkyl; and with the further proviso that R_3 is hydrogen when R_1 and R_2 taken together form oxo, with a salt of aradioactive metal.

23. A process for the preparation of a compound of the formula



wherein R_{10} is -OH or R_6-NH-; R_6 is lower alkyl R_1 and R_2 are individually hydrogen or lower alkyl or taken together form oxo; R_8 is hydrogen or an amino protecting group; R_4 is hydrogen or lower alkyl; R_5 is hydrogen or a thiol protecting group and x and y are integers from 0 to 2 with the proviso that when R_1 and R_2 form oxo, R_8 is hydrogen, which comprises

a) for the preparation of a compound wherein R_{10} is -OH treating a compound of formula



I

wherein R is hydrogen or lower alkyl, R_1 and R_2 are individually hydrogen or lower alkyl or taken together form oxo; R_3 is an amino protecting group where R_1 and R_2 are individually hydrogen or lower alkyl; R_3 is hydrogen when R_1 and R_2 taken together form oxo; R_4 is hydrogen or lower alkyl; R_5 is hydrogen or a thiol protecting group; x and y are integers from 0 to 2,

with a base or

b) for the preparation of a compound wherein R_{10} is R_6-NH- treating a compound of formula I above with a

compound of formula

$$R_6 - NH_2 \qquad X$$

wherein R_6 is as above.

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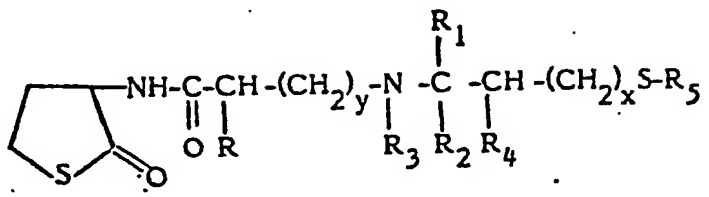
1933

Claims for Austria 4090/141

1. A process for the preparation of a compound of the formula

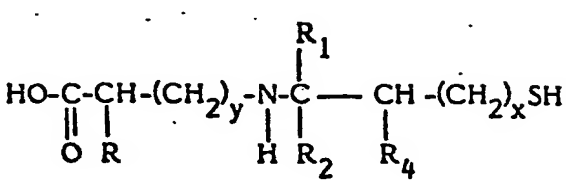


I

wherein R is hydrogen or lower alkyl; R_1 and R_2 are individually hydrogen or lower alkyl or taken together form oxo; R_3 is an amino protecting group where R_1 and R_2 are individually hydrogen or lower alkyl; R_3 is hydrogen when R_1 and R_2 taken together form oxo; R_4 is hydrogen or lower alkyl, R_5 is hydrogen or a thiol protecting group; and x and y are integers from 0 to 2,

which comprises

a) reacting a compound of formula

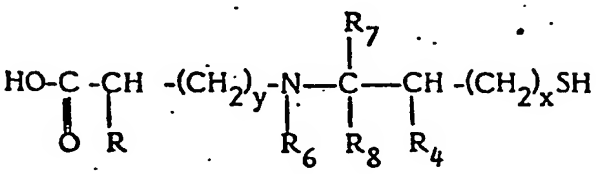


II

wherein R, R_1, R_2, R_3, R_4, x and y are as above,

or

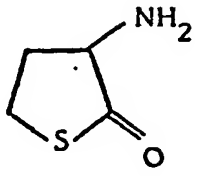
b) reacting a compound of formula



II-A

wherein x, y, R and R_4 are as above; R_6 is an amino protecting group; R_7 and R_8 are individually hydrogen or lower alkyl,

with a compound of formula



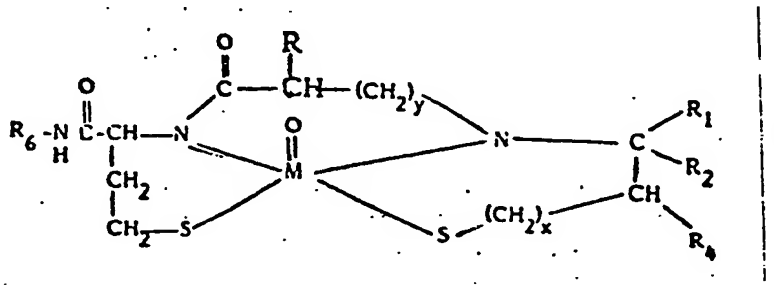
IV

2. A process as claimed in claim 1, wherein N-(t-butyloxycarbonyl), N-(2-mercaptoethyl)glycyl homocysteine thiolactone is prepared.

3. A process as claimed in claim 1, wherein N-[2-(S-acetamidomethyl)mercaptopropionyl]glycyl homocysteine thiolactone is prepared.

4. A process as claimed in claim 1, wherein N-[2-(S-benzamidomethyl)mercaptopropionyl]glycyl homocysteine thiolactone is prepared.

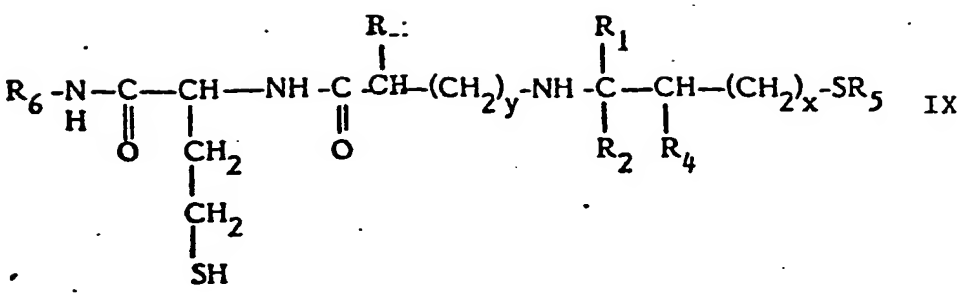
5. A process for the preparation of an anionic chelate of the formula



VI

wherein R_6 is lower alkyl; R_1 and R_2 are individually hydrogen or lower alkyl or taken together form oxo; M is a radioactive metal; R_4 is hydrogen or lower alkyl; x and y are integers from 0 to 2

or a salt of said anionic chelate, which comprises reacting a compound of the formula



IX

wherein R, R_1, R_2, x, y, R_3, R_4, R_5 and R_6 are as above, with the proviso that R_3 is an amino protecting group when R_1 and R_2 are individually hydrogen or lower alkyl; and with the further proviso that R_3 is hydrogen when R_1 and R_2 taken together form oxo, with a salt of aradioactive metal.

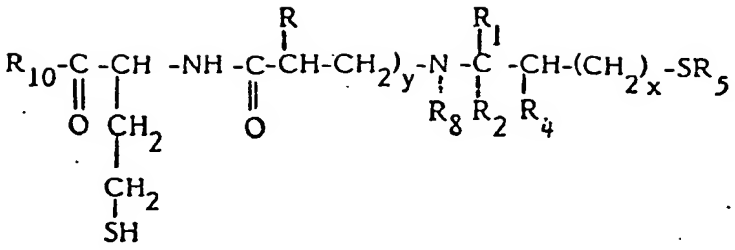
6. A process as claimed in claim 5 wherein M is technetium-99m.

7. A process as claimed in claim 5, wherein [2-[[1-(2-mercaptoethyl)-2-(methylamino)-2-oxoethyl]amino]-N-(2-mercaptoethyl)glycinato N,N',S,S']oxotechnetate-99m is prepared.

8. A process as claimed in claim 5, wherein said chelate is prepared in the form of its alkali metal salt, ammonium salt or amine salts.

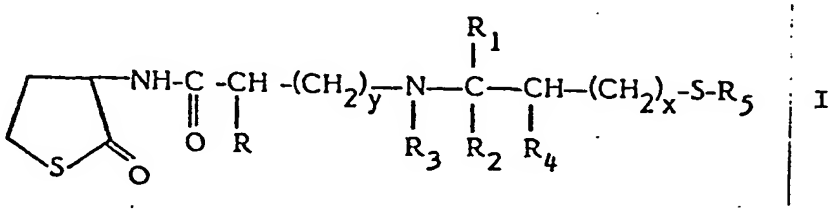
9. A process as claimed in claim 5, wherein [1-[[1-(2-mercaptoethyl)-2-(methylamino)-2-oxoethyl]amino]-N-(2-mercapto-1,1-oxopropyl)glycinato-N,N',S,S']oxotechnetate-99m is prepared.

10. A process for the preparation of a compound of the formula



wherein R_{10} is -OH or R_6-NH-; R_6 is lower alkyl R_1 and R_2 are individually hydrogen or lower alkyl or taken together form oxo; R_8 is hydrogen or an amino protecting group; R_4 is hydrogen or lower alkyl; R_5 is hydrogen or a thiol protecting group and x and y are integers from 0 to 2 with the proviso that when R_1 and R_2 form oxo, R_8 is hydrogen, which comprises

a) for the preparation of a compound wherein R_{10} is -OH treating a compound of formula



I

wherein R is hydrogen or lower alkyl, R_1 and R_2 are individually hydrogen or lower alkyl or taken together form oxo; R_3 is an amino protecting group where R_1 and R_2 are individually hydrogen or lower alkyl; R_3 is hydrogen when R_1 and R_2 taken together form oxo; R_4 is hydrogen or lower alkyl; R_5 is hydrogen or a thiol protecting group; x and y are integers from 0 to 2,

with a base or

b) for the preparation of a compound wherein R_{10} is R_6-NH- treating a compound of formula I above with a compound of formula

$R_6 - NH_2$ X

wherein R_6 is as above.

11. A process as claimed in claim 10, wherein a compound of claim 10 is prepared in which R_5 is hydrogen.

12. A process as claimed in claim 10, wherein N-(t-butyloxycarbonyl), N-(2-mercaptoethyl)glycyl N'-methyl-homocysteinamide is prepared.

13. A process as claimed in claim 10, wherein N-(2-mercaptoethyl)gycyl N'-methylhomocysteinamide is prepared.

14. A process as claimed in claim 10, wherein a compound is prepared in which R is a thiol protecting group.

15. A process as claimed in claim 10, wherein N-[2-(3-acetamidomethyl)mercaptopropionyl]glycyl N-methyl-homocysteinamide is prepared.

16. A process as claimed in claim 10, wherein N-[2-(S-benzylamidomethyl)mercaptopropionyl]glycyl N-methyl-homocysteinamide is prepared.

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